Limitations of Automated Remnant Lipoprotein Cholesterol Assay for Diagnostic Use

To the Editor:

I wish to comment on the limitations of an automated remnant lipoprotein cholesterol (RemL-C) assay reported in Clinical Chemistry (1). Remnants are lipoprotein particles produced after newly formed triglyceride-rich lipoprotein particles of either hepatic or intestinal origin enter the plasma space and undergo lipolysis via the action of lipoprotein lipase in the capillary bed. During this process, these lipoproteins lose triglyceride and pick up cholesteryl ester and apolipoprotein E (apoE) from other lipoproteins through the action of cholesteryl ester transfer protein. The development of a clinical diagnostic method for measuring remnant lipoprotein cholesterol has been hampered by difficulties with isolation. Moreover, the characteristics of remnant lipoproteins have not been clearly defined. The most consistent definition of remnant lipoproteins has been proposed as an apoE-rich lipoprotein fraction within TRLs, which increases in the postprandial state. The original immunoseparation method for the measurement of remnant-like lipoprotein particle cholesterol (RLP-C) was developed by Nakajima and colleagues in Japan and satisfied these criteria. Normal ranges in the US for this analyte were developed using samples from the Framingham Offspring Study (2). The separation of newly formed TRLs from remnant lipoproteins is crucial because the later lipoproteins are atherogenic and are important for the assessment of coronary heart disease (CHD) risk, especially in women.

My colleagues and I previously reported that an increased RLP-C, in contrast to triglyceride, was a significant independent predictor of prospective CHD in female participants in the Framingham Offspring Study, after adjustment for other standard risk factors including LDL and HDL cholesterol concentrations (3). In addition, in this study triglyceride values correlated with RLP-C ($r = 0.79, P < 0.001$), but the correlation was not strong enough to affect the clinical utility of the RLP-C assay. We also recently measured remnant lipoprotein cholesterol with the RemL-C assay as described (1) in samples from 3201 male and female participants in cycle 6 of the Framingham Offspring Study. We noted a highly significant correlation between these values and triglyceride concentrations ($r = 0.93, P < 0.0001$). Moreover, values obtained with this assay were not independent predictors of CHD in either a case-control or prospective fashion. These data suggest that this recently developed automated assay for remnant lipoprotein cholesterol does not accurately measure the cholesterol content of remnant lipoproteins, but rather total TRLs. This issue has taken on increasing importance since prospective studies have reported that postprandial triglyceride (TG) concentrations are associated with significantly

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Clinical Chemistry 55:11 (2009) 2061
higher risk of CHD than fasting TG concentrations, suggesting that such differences can be due to postprandial increases in remnant lipoproteins (4). Nakajima et al. (5) have recently reported that 80% of the increase in triglyceride concentrations in the postprandial state results from increases in remnant lipoproteins using the RLP assay. Therefore, postprandial TG may reflect higher CHD risk than fasting TG due to increased remnant lipoproteins. These data also indicate that the correlation between remnant lipoproteins and TG should be different in the fasting and postprandial states, namely a higher correlation between remnant lipoproteins and TG in the postprandial state. However, the RemL-C assay did not show such differences between TG vs fasting and postprandial RemL-C in our other studies.

These two different remnant assay methods (RLP-C and RemL-C) have been approved for the measurement of serum remnant lipoprotein cholesterol concentrations in Japan, whereas only the RLP-C assay is approved for this purpose in the US. The benefits of the newer RemL-C assay are that it does not require pretreatment and can be readily used on an automated analyzer. In my view, however, this latter assay has limitations because of its very high correlation with triglyceride concentrations and its lack of CHD risk prediction in the Framingham Offspring Study, suggesting a lack of specificity for remnant lipoproteins.

Letters to the Editor

Authors’ Disclosures of Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References


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Previously published online at
DOI: 10.1373/clinchem.2009.133934

In Reply

We appreciate the concerns from Ernst J. Schaefer on our article regarding development of a homogeneous assay for remnant lipoprotein cholesterol (RemL-C)1 (1). I agree that RemL-C has a limitation for a diagnostic use in a certain situation. The process of this assay includes a degradation step of lipoproteins by detergents. Freeze-thawing or long-term storage, which also degrades lipoproteins, yields a nonnegligible effect on the values measured by RemL-C. This fact limits the use of RemL-C assay to fresh samples or samples frozen and thawed only once. I am afraid that samples from the Framingham Offspring Study were measured after repeated freeze-thaw or long-term storage.

Schaefer defined remnant lipoproteins in the concerned letter as the apolipoprotein E-rich lipoprotein fraction within triglyceride-rich lipoproteins. RemL-C reagents did surely react to these lipoproteins as shown in the original article (1). Schaefer mentioned that RemL-C correlates with triglyceride (TG) too closely to be employed for clinical use. Recent reports, however, clearly demonstrated that RemL-C has value in detecting a high-risk group for atherosclerosis. Nakada et al. (2) reported that RemL-C concentrations were increased in patients with coronary artery disease independent of TG concentrations, and they concluded that the RemL-C assay has a clinical significance in assessing coronary risk, particularly among patients with normal TG concentrations. In another study, Yoshino et al. revealed that RemL-C did not always correlate with TG, especially in patients with diabetes mellitus (personal communication, G. Yoshino, 2062 Clinical Chemistry 55:11 (2009)